A DESCRIPTION OF THERMOPHILIC ACTINOMYCETES CULTIVATED FROM CHAMPIGNON COMPOST

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Received on October 15th, 1969

For a long time data on the systematization of thermophilic actinomycetes were rather scauty, and even now no unambiguous and clear system is to be found in the literature. The first comprehensive systems of actinomycetes (Waksman, 1939; Bergey, 1948; Krassilnikov, 1949) were based mainly on morphological properties. In his work Misusstin (1950) has presented a separate table which facilitated the determination of actinomycetes. Kossmatsev (1953) emphasized that the thermophilic actinomycetes should get an independent place in the system of actinomycetes. In one of the most up-to-date systems of thermophilic bacteria (Jacob, 1961) the thermophilic actinomycetes can be found under the genera *Streptomyces* and *Micromonospora*, respectively. The species isolated and identified by Henssen (1957), too, are incorporated in Waksman's (1957) system under a separate series (Thermophilus XVI. series). On the basis of the characteristic formation of sporophores, Krassilnikov and Agree (1964) have established e new genus named *Actinobifida*. Preobraskensskaja (1966) investigated the infra-red spectrum of the spores of actinomycetes. In her opinion this property serves well the purpose of systematization. The working system of Gibbs and Skinner (1966), though based on current systems, facilitates both the identification and determination of the strains. Desai and Dhala (1967) have introduced a new species, the so-called *Streptomyces thermonitrificans*. Krassilnikov et. al (1968) again have described three new species of thermophilic actinomycetes named *Actinomyces macrosporus*, *Actinomyces megasporus*, and *Actinomyces glaucosporus*. In their general work, Hubert et al. (1967) have established several types of actinomycetes according to cell wall composition, and suggested corresponding modifications.

In the present paper the determination of some thermophilic actinomycetes cultivated from champingon compost is described.

Materials and methods

Description of the test material and the method of isolating actinomycetes strains can be found in a previous work (B a l l a , 1968). In the determinations of the strains the following tests have been carried out:

1. *Macromorphological tests*

a) Cultivation properties in solid culture medium

The rate of growth, colony formation as well as the amount and colour of the vegetative and aerial mycelia have been investigated on glucose-asparagine agar, pepton-beef extract or nutrient agar (pepton agar), respectively, in Petri-dishes.

b) Pigment formation

As a culture medium potatoe slices were used (W a k s m a n, 1961). G r e i n and K ü s t e r (1955) have emphasized the importance both of the state of health and the variety of the potatoes used for experimental purposes.

2. *Biochemical reactions and some physiological properties*

The test methods and materials (culture media and reagents) applied have been described by H o r v á t h (1965), the abbreviations are also taken from the above work.

a) Liquefaction and decomposition of gelatin (Gelatinase activity)

The liquefaction of gelatin was investigated in a culture placed in high nutrient (T 3), and its complete decomposition on Frazier-agar (T 22). The exoenzyme gelatinase has been tested by means of tannin solution (R 25).

The presence and size of the light spot observed around the colony was indicative as to the extent of decomposition (Figs. 1.—2.).

b) Nitrate reduction

A nitrate brot with Durham's tube (T 36) was applied, and the products that formed during the process were demonstrated by nitrate—(R 24) nitrite—(R 25) and Nessler reagents (R 30), respectively.

c) Effects of actinomycetes on milk (coagulation, peptonization)

The changes occurring in the consistency of milk and the shift in the pH were investigated in a litmus-milk medium (T 43).

d) Casease activity

On a milk-agar culture medium (T 25) the light zone developing around the colonies indicates the enzymatic hydrolysis of casein, and the extent of decomposition can be determined from the size of this zone (Figs. 3—4).

e) Amylase activity (starch decomposition)

On starch-agar (T 27) plates the presence of the extracellular enzyme has been established by means of the well known iodine (Lugol solution R 19) – starch colour reaction. Measurement of the size of the decomposition zone offered approximate, comparative data as to the extent of decomposition.

3. Effects of environmental conditions

a) Effect of temperature

The rate of growth was investigated on pepton-agar (Waksman, 1961) in Petri-dishes, at various temperatures (24° – 65°C).

b) Type of respiration

In these tests a liquid culture medium (T 1) and the catalase test were applied.

4. Antibiotic effect

The antagonistic effect of actinomycetes strains was investigated by means of four test microorganisms *(Proteus vulgaris, Escherichia coli, Staphylococcus aureus,* and *Saccharomyces cerevisiae).* With the cultures in Petri-dishes the hole and vital point test methods were applied.

5. Micromorphology

Microscopic examination of thermophilic actinomycetes strains was followed by the hanging drop method in yeast-glucose and starch nutritive broth (Waksman, 1961). The diameter of the vegetative and aerial mycelia and that of the spores, the mycelium branches, the forms of spore formation and the size of spores, respectively, were measured, and photographed.

Results and conclusions

Upon complete testing and evaluation of macromorphological, biochemical, physiological and micromorphological properties of the 73 isolated pure thermophilic actinomycetes strains the following actinomycetes species have been identified (Waksman's system, 1961):

1. *Streoptomyces thermoviolaceus* Henssen ssp. *apingens* Henssen, 1957

Morphological description: The vegetative mycelia are nonseptate, they form closely packed flocks and are slightly branched (cf. Fig. 7), the aerial mycelia are often twisted (Fig. 8), the sporophores are curved and 16 – 24 – 40 μ in lenght, they turn completely into spores. The spores are formed in chains, they are oval-shaped and 1.0 – 1.6 μ in size (Fig. 9).

Cultivation properties:

a) On pepton agar: fair growth , the colonies are round and bulging, aerial mycelium formation is abundant from white to deep grey in colour.

b) On glucose-asparagine agar: intensive growth, the colonies are round and flat, aerial mycelium formation is abundant from white to deep grey in colour.

c) On potato culture medium: fair growth, aerial mycelium formation only in patches, white, pigment is excluded.

Occurrence: in fresh horse or pig manure and in the composts made of them.

The results on the biochemical and physiological properties are summarized in Table 1.

2. *Streptomyces thermodiastaticus* (Bergey, 1919) Waksman et al., 1939

Morphological description: The vegetative mycelia are nonseptate, branched and they form closely packed flocks (Fig. 10), the aerial mycelia are branched and the sporophores developed from them exhibit a spiral shape (Fig. 11) and turn completely into spores. The spores are formed in chains, their shape varies from round to oval and they are $0.7-0.9\ \mu$ in size (Fig. 12)

Cultivation properties

a) On pepton agar: moderate growth, round flat colonies, the white aerial mycelium is well developed.

b) On glucose-asparagine agar: fair growth, the colonies are round and bulging, abundant aerial mycelium formation from white to grey in colour.

c) On potato culture medium: moderate brownish yellow growth, aerial mycelium formation in patches, the colour is light grey, pigment excluded.

Occurrence: in soil and compost.

3. *Streptomyces thermofuscus* Waksman et al., 1939

Morphological description: The vegetative mycelia are nonseptate and the aerial mycelia formed from them are ascending (Fig. 14), the shape of the sporophores varies from straight to spiral (Fig. 15). The spores are formed in chains and are $0.8-2.0\ \mu$ in diameter and round (sometimes slightly spheroid) in shape (Fig. 16).

Cultivation properties

a) On pepton agar: intensive growth, the colonies are round, the dark grey aerial mycelium formation is abundant.

b) On glucose-pepton agar: moderate growth, the colonies are round with toothed edges, poor grey aerial mycelium formation.

c) On potato culture medium: intensive brownish yellow growth, white aerial mycelium in patches, soluble black pigment is involved.

Occurrence: in horse manure and in the compost made from it.

Table I.

Biochemical and physiological properties of thermophilic actinomycetes

Species	I.		II.	III.			IV.		V.		VI.	VII.	
	1	2		1	2	3	1		2			1	2
							a	b	a	b			
Streptomyces thermoviolaceus ssp. apingens	+	+	–	+++	+++	++	+++		+		–	50 – 55°	24 – 60°
Streptomyces thermodiastaticus	+	+	+	–	–	–	+++	+			–	55°	24 – 60°
Streptomyces thermofuscus	++	++	+	++	++	++	+++				–	55°	24 – 65°
Thermomonospora lineata	+	+	+	+	+	+	+++	+			–	55°	37 – 60°
Thermoactinomyces vulgaris	+	+	–	++	++	++	+++				–	55°	24 – 60°
Thermoactinomyces thermophilus	+	+	–	+	+	+	+++		+		–	50°	37 – 60°

Symbols: I. = liquefaction (1) and decomposition (2) of gelatin
II. = nitrate reduction
III. = milk: coagulation (1), peptonization (2) casein decomposition (3)
IV. = starch decomposition
V. = type of respiration: aerob (1) – facultative (a)
– obligate (b)
anaerob (2) – facultative (a)
– obligate (b)
VI. = antagonistic effect
VII. = temperature: optimum (1), range (2)
Strength of reaction: strong (+++)
mediocre (++)
poor (+)
none (–)

4. *Thermomonospora lineata* H e n s s e n, 1957

Morphological description: The vegetative mycelia are nonseptate and branched, the aerial mycelia are the most branched among the Thermomonospores (Fig. 17). The sporophores are straight, the spores are formed on simple or on branched sporophores, the spore masses are hair-like or knotted (Fig. 18). The spores are round and $0.8-1.5$ μ in size (Fig. 19).

Cultivation properties

a) On pepton agar: fair growth, round and bulging colonies, abundant white aerial mycelium formation.

b) On glucose-asparagine agar: fair growth, round but flat colonies, mediocre white aerial mycelium formation.

c) On potato culture medium: poor growth, white aerial mycelium in patches, pigment is excluded.

Occurrence: in animal manure composts.

5. *Thermoactinomyces thermophilus* (B e r e s s t n e v, 1897) W a k s m a n et al., 1939

Morphological description: The vegetative mycelia are nonseptate, thickly branched and form closely packed flooks (Fig. 20); The aerial hyphae are either simple or branched, they are formed from terminal or side branches of the vegetative mycelia, the sporophores are straight (N o a c k, W a k s m a n) (Fig. 21) or curved, they often exhibit a spiral shape (K r a s s i l n i k o v) (Fig. 22). The spores are round (lightly spheric) and remain together in the chains (Fig. 23).

Cultivation properties

a) On pepton agar: fair growth, the vegetative colonies are round, flat and exhibit a yellowish brown colour, the aerial mycelium formation is abundant from white to dark grey.

b) On glucose-asparagine agar: fair growth, the yellow vegetative colonies are round and flat, the white-dark-grey aerial mycelium formation is mediocre.

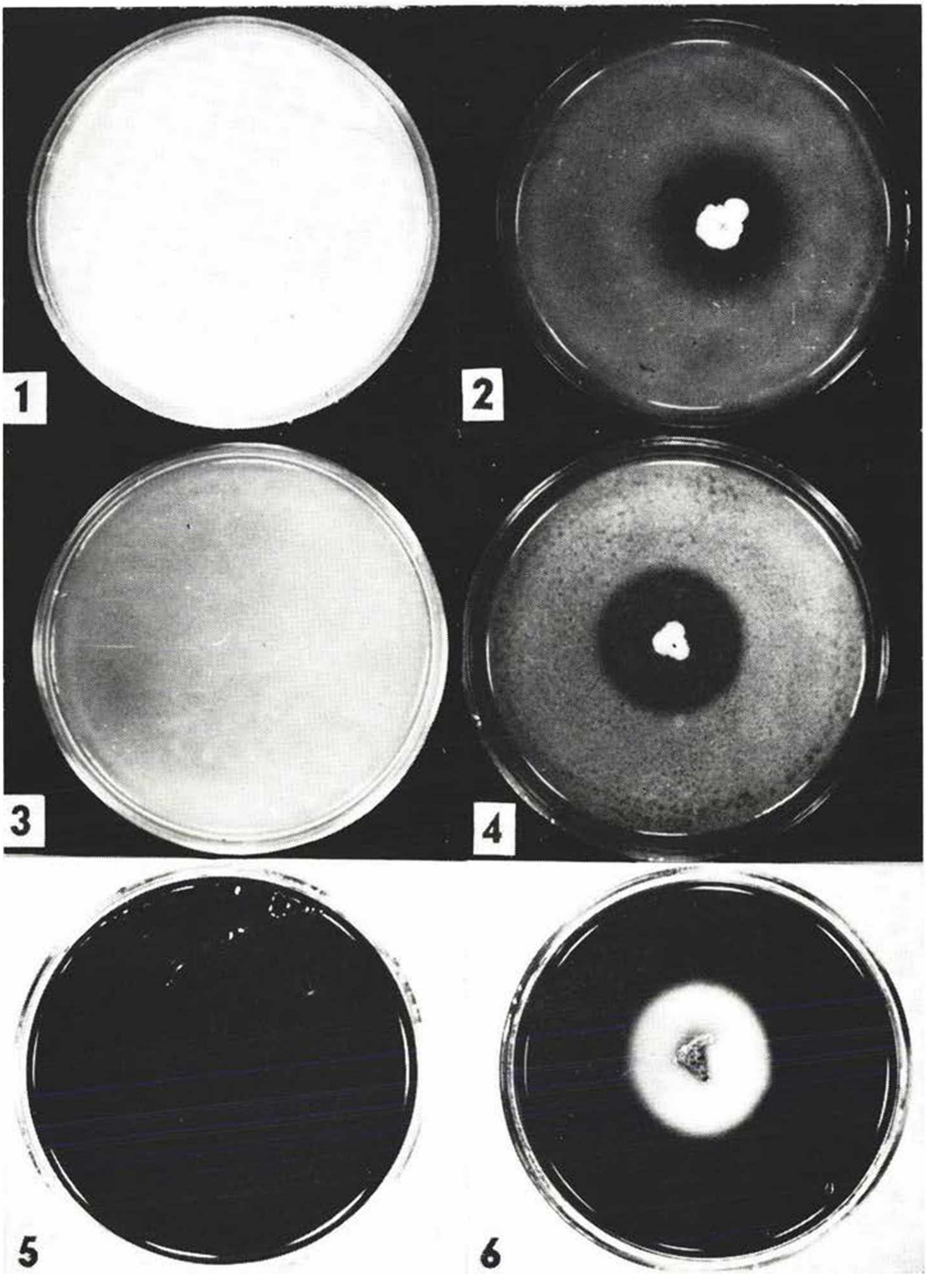
c) On potato culture medium: fair growth, the vegetative colonies are yellowish brown, poor greyish-white aerial mycelium formation in patches, soluble brown pigment involved.

Occurrence: in soil and in animal manure composts.

6. *Thermoactinomyces vulgaris* T s i k l i n s k y 1899

(Synonim: *Micromonospora vulgaris* W a k s m a n et al., 1939)

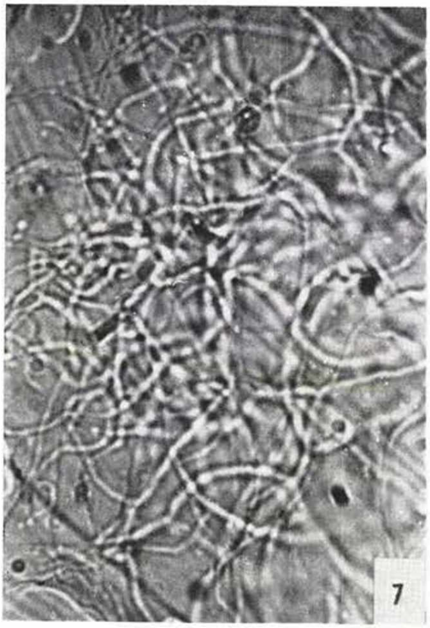
Morphological description: The vegetative mycelium is nonseptate and slightly branched, 0.5 μ in diameter (Fig. 24). The spores, round or oval in shape, are formed one by one at the end of the mycelium branches that is to stay at the end of the short branches from where they are likely to get torn off. Frequently, the spores are sitting right upon the mycelium (Fig. 26).

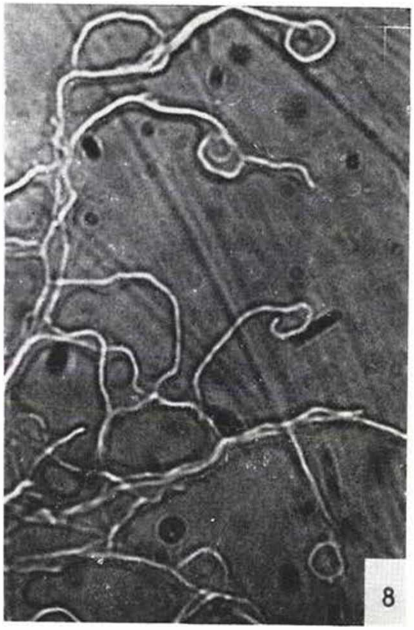


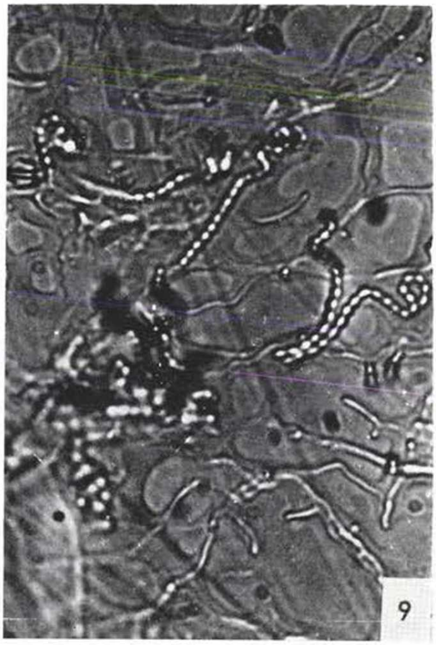
Figs. 1 – 2. Control and decomposition of gelatin

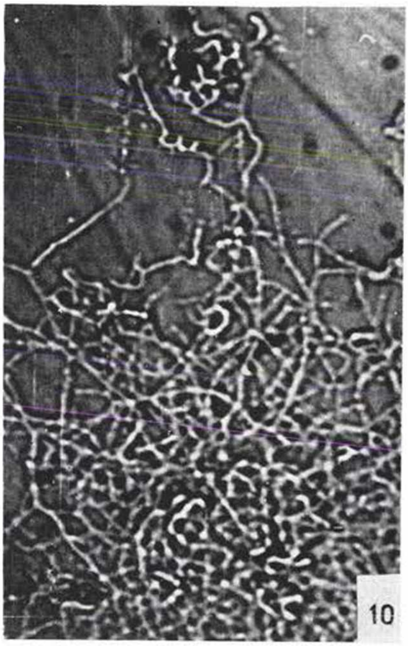
Figs. 3 – 4. Control and decomposition of casein

Figs. 5 – 6. Control and decompositionof starch





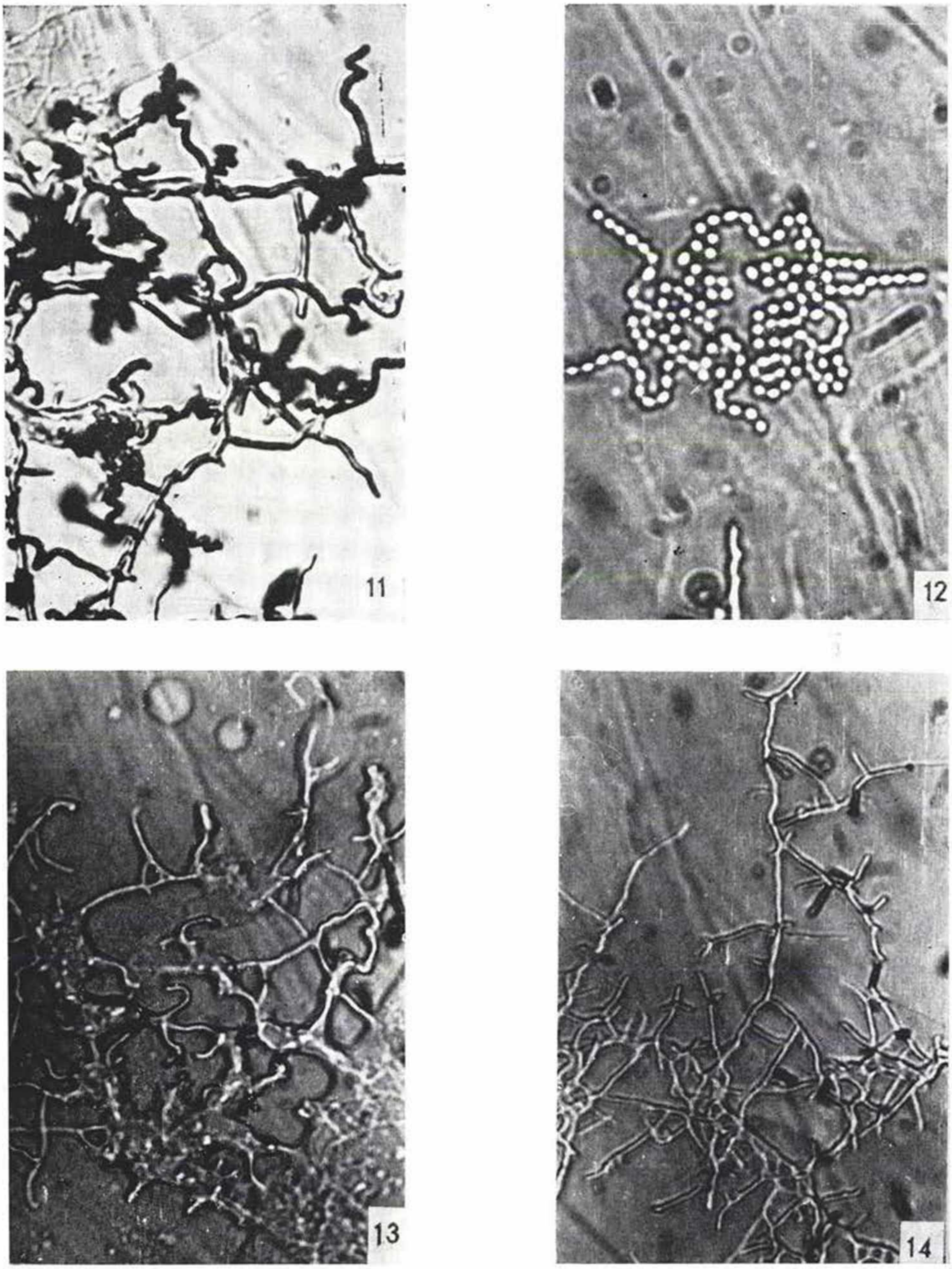




Figs. 7 – 8. Vegetative and aerial mycelia of *Streptomyces thermoviolaceus* ssp. *apingens*. M = 1000:1

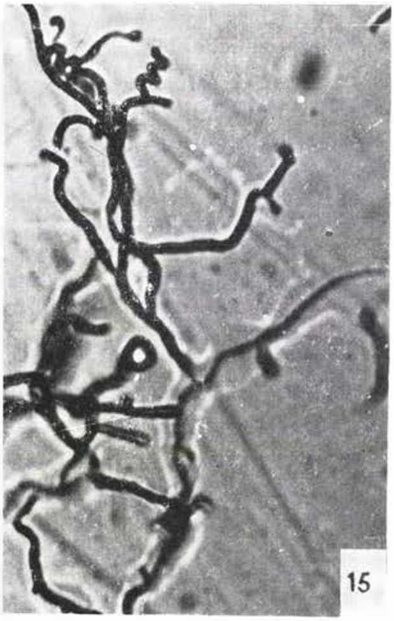
Fig. 9. Spores of *Streptomyces thermoviolaceus* ssp. *apingens*. M = 700 : 1

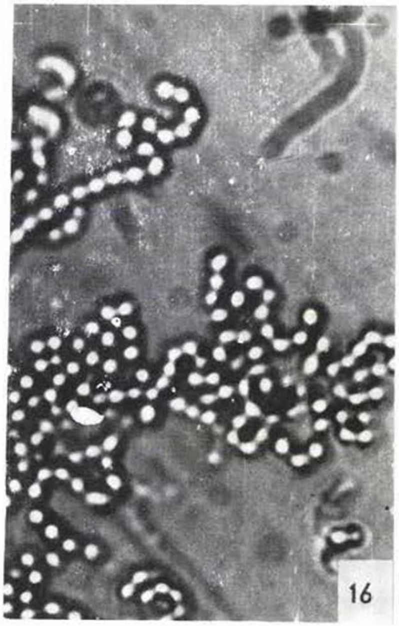
Fig. 10. Vegetative mycelia of *Streptomyces thermodiastaticus*. M = 600 : 1

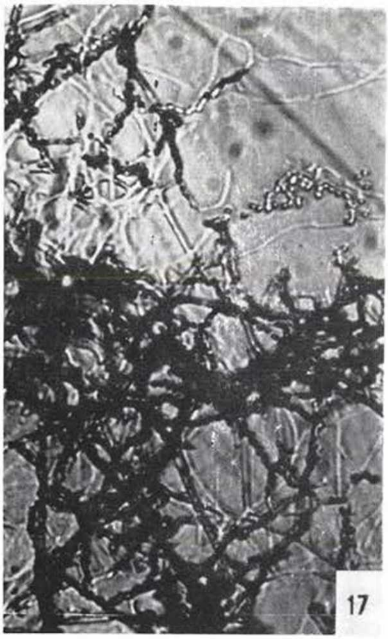


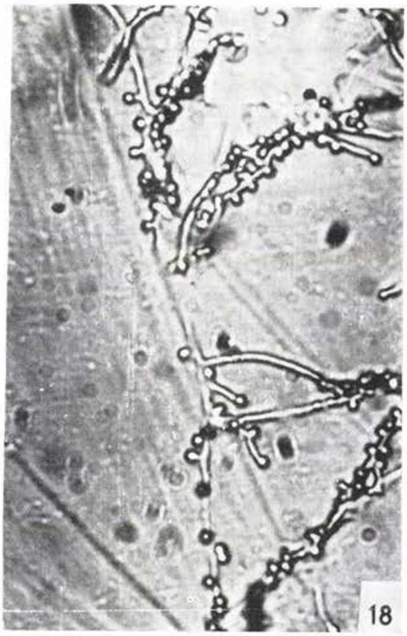
Figs. 11 – 12. Aerial mycelia and spores of *Streptomyces thermodiastaticus*. M = 1400 : 1

Figs. 13 – 14. Vegetative and aerial mycelium formation of *Streptomyces thermofuscus*. M = 500 : 1





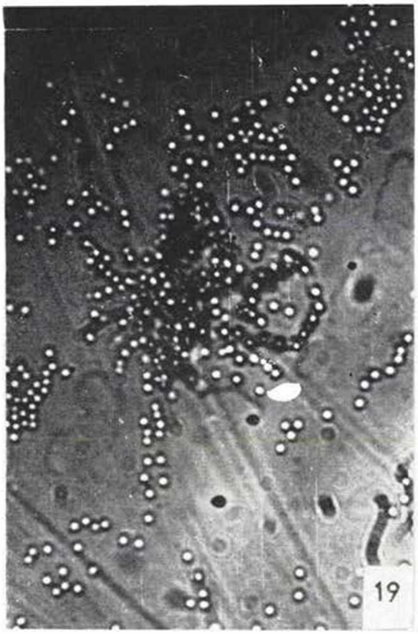


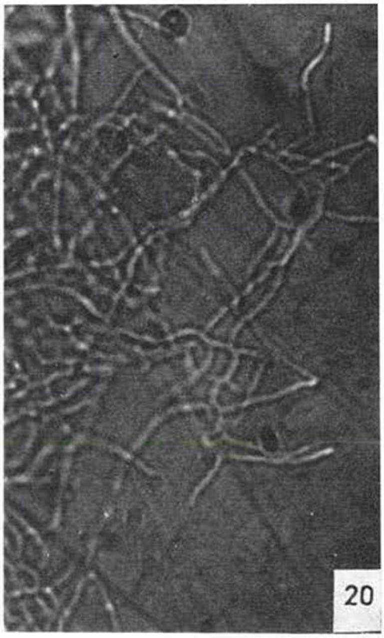


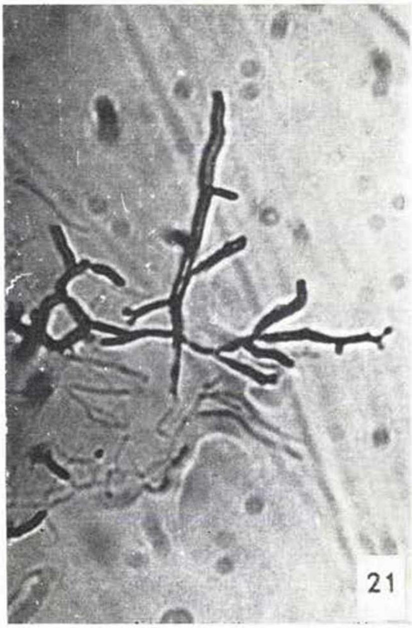
Figs. 15 – 16. Sporophores and spores of *Streptomyces thermofuscus*. M = 1400 : 1

Fig. 17. Vegetative and aerial mycelia of *Thermomonospora lineata*. M = 500 : 1

Figs. 18 – 19. Sporophores and spores of *Thermomonospora lineata*. M = 900 : 1







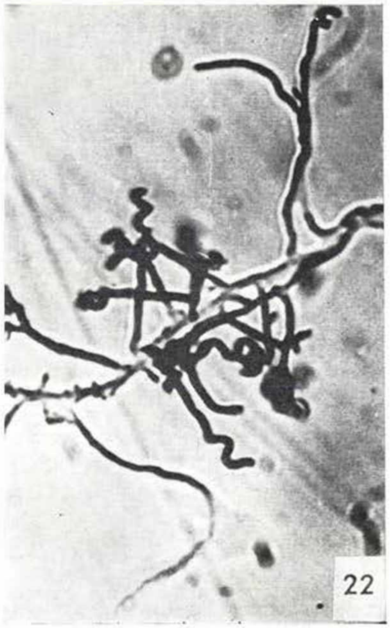
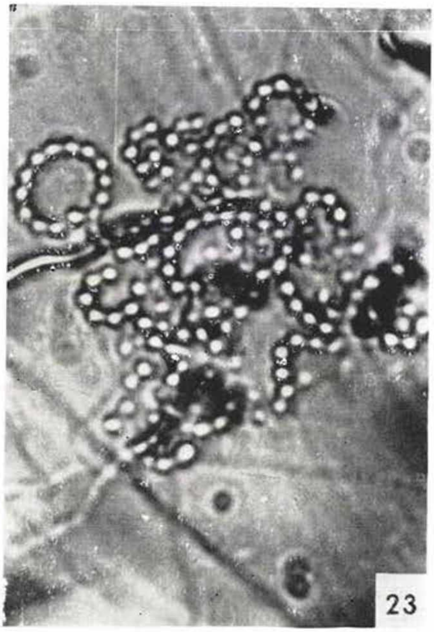
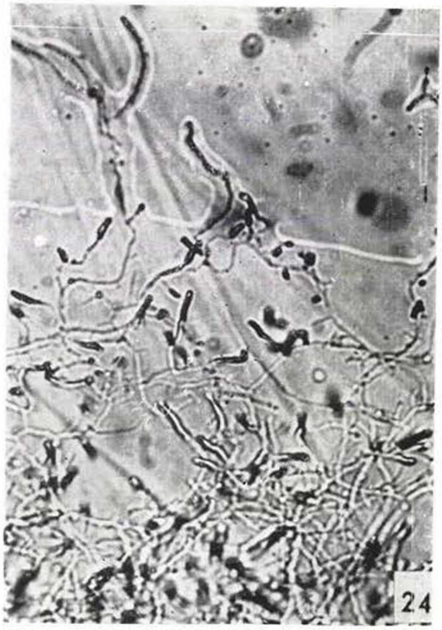
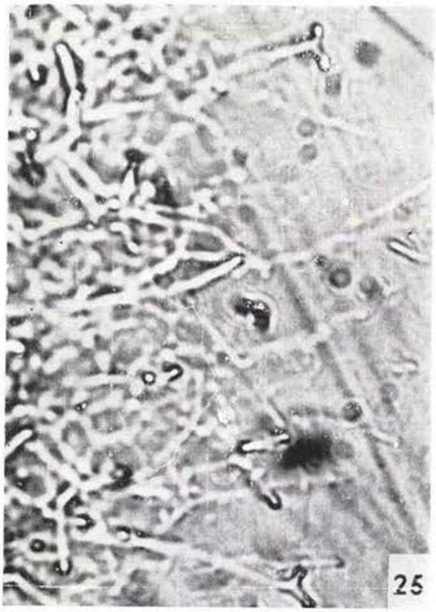


Fig. 20. Vegetative mycelia of *Thermoactinomyces thermophilus*. M = 500 : 1

Figs. 21 – 22. Aerial mycelia and sporophores of *Thermoactinomyces thermophilus*. M = 1000 : 1







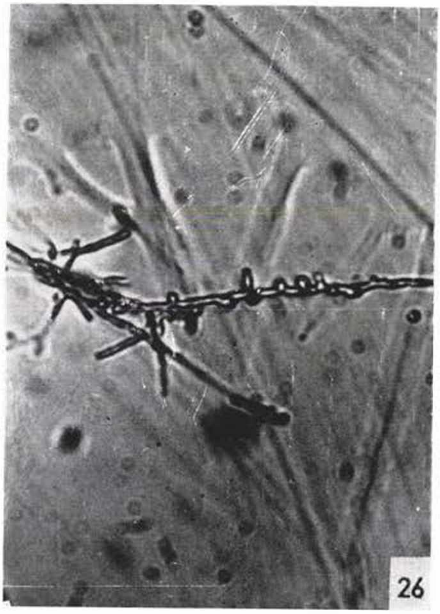


Fig. 23. Spores of *Thermoactinomyces thermophilus*. M = 1200 : 1

Fig. 24. Vegetative mycelia of *Thermoactinomyces vulgaris*. M = 600 : 1

Figs. 25 – 26. Forms of spore formation of *Thermoactinomyces vulgaris*. M = 1000 : 1

Cultivation properties

a) On pepton agar: intensive growth, round an bulging colonies, the greyish white aerial mycelium formation is abundant.

b) On glucose-asparagine agar: fair growth, the brownish yellow vegetative colonies are round and flat, poor white aerial mycelium formation is involved.

c) On potato culture medium: mediocre growth, the vegetative colonies are light yellow, poor white aerial mycelium formation in patches, pigment is excluded.

Occurrence: in human and animal excrements, hay, soil and composts of high temperature.

Conclusions

1. The 73 isolated thermophilic actinomycetes strains proved to be the representatives of 9 species belonging to different genera.
2. As the morphological properties described in the prevailing system rather overlap one another, they scarcely suit the purpose of isolation.
3. In the systematization much more importance must be attached to the investigation of biochemical and physiological properties.
4. All these as well as the recent data reported in the literature (H u b e r t et al., 1967) underline the importance of establishing a final system of thermophilic actinomycetes.

Summary

In order to determine the pure thermophilic actinomycetes strains isolated from champignon compost the following investigations have been carried out:

macromorphological tests
comparative tests on biochemical and
some physiological properties
investigations on the effects of enviromental
conditions and antibiotics
micromorphological tests

According to the experimental results and to the systematization scheme of W a k s m a n (1961), the 73 strains investigated proved to be the representatives of 9 species belonging to different genera.

Three thermophilic actinomycetes species have been described in a previous paper (B a l l a, 1968).

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